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Comment

CO III gene in bacteria

'Homology between bacterial DNA and bovine mitochondrial DNA encoding cytochrome *c* oxidase subunit III' by Pamela S. Fink, Tracy Whitford, Michael Leffak and Lawrence J. Prochaska [(1987) FEBS Lett. 214, 75–80]

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In a recent *Research Letter*, Fink and her collaborators [1] discuss the occurrence of genes in prokaryotes that would be homologous to the subunit III (CO III) gene of the mitochondrial cytochrome oxidase.

The experiment is carried out as follows. The authors clone a fragment from bovine mtDNA that contains 70% of the CO III gene and use this clone as a hybridization probe. DNA from several bacteria that are known to possess the homologous enzyme (cytochrome *aa*) is extracted, digested with restriction enzymes and studied by Southern blotting. The authors fail to obtain any hybridization under the standard conditions (65°C) and so lower the stringency (temperature) in the reaction to get the probe to bind to the template. Some of their samples bind the probe at 45°C while others show clear binding only at 25°C. In all cases the probe binds to the smear of restriction fragments almost uniformly: no clear bands are formed.

Cross-hybridization, that is the use of a DNA probe obtained from one species for detection of a gene in another, is often a difficult experiment. Moreover, mtDNA with its unique genetic code is a particularly difficult source for probes that are going to be used with bacterial DNA.

If the homology between the probe and the target gene is low, it is hard to get a signal in cross-hybridization experiments. 'Getting a signal' means that a defined band or a couple of bands are seen instead of continuous radioactivity along the electrophoresis lanes in the autoradiograms [1]. The latter result is normally regarded a 'background'. The experiment searches for a hilltop over a silhouette of forest; if only the latter becomes visible, the experiment fails.

Fink et al. do not see this simple fact but use the slight differences in the level of background to discuss the presence or absence of the CO III genes in different bacteria. This discussion is completely artificial.

The only bacterium in which the CO III gene is known to be is *Paracoccus denitrificans* [2]. In the experiment of Fink et al. the DNA fragments from this bacterium give, however, 'weak' hybridization. This is a situation that should have alarmed the authors and not encouraged them to discuss 'weaker' and 'stronger' homologies between the bovine mitochondrial and the bacterial CO III genes [1].

The widespread use of DNA methods in biochemistry is relatively young. But this is no excuse to the experimentors to be blind to these methods when they want to use them.

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